Maronpot, Robert R. 2004

Dr. Robert R. Maronpot Oral History 2004

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Dr. Robert Maronpot

Shostak: Great. So you said, overall….

Maronpot: Many years ago, the immunology folks realized there were B cells and T cells, and different classes of antibodies, and they proposed that the development of this knowledge/technology that they had discovered would answer lots of questions, perhaps most important, questions about cancer. And there was a lot of hype. In the end it didn't answer anywhere near as much as promised, but ended up being a very valuable tool to put in the toolbox to be used when appropriate.

And then, after some years, there was the discovery of oncogenes and tumor-suppressor genes, and there was a lot of hype about that. We were going to be able to understand key critical elements, at least as applied to cancer. And after enough testing it was realized that there was a lot of diversity in response. Oncogenes didn't answer all the questions that had been promised, but became another tool to get put in the toolbox,hopefully to be removed and used when appropriate.

And then transgenics came along, and there were a lot of promises there, including getting quicker answers to carcinogenic potential. Big validation studies of international scope were undertaken. NIEHS was involved and ILSI as well as a lot of other organizations. And when the validation studies were over, it wasn't quite as good as people had thought it was going to be. A couple of surprises came along, but it turned out that using transgenic mice wasn' t getting you anything better than what you really had before for identifying carcinogenic potential by animal testing. Hopefully this approach has been put in the toolbox to be removed and used when appropriate. And this is a very complex tool because there are so many different genetically engineered animals.

And now we're facing toxicogenomics and proteomics, and there's a lot of hype and promise that this is going to be the ultimate in biomarkers to identify, not just cancer now, but just about anything that one might want to identify.

Well, the hype is still there because the technology is new, and it's at that stage where the technology is actually driving things more than the question or the goal. I predict that in the end, toxicogenomics and proteomics will be tools to be put in the toolbox to be removed and used when appropriate.

This is the overview perspective that I have, and it seems to be confirming itself. I understand why people put the hype behind it, particularly the academics. That's how they get their grants. Consider this as a backdrop underlying answers to specific things you might ask.

Shostak: Okay. That's great. Can I ask you one question about what you just said? You said that in some ways the technology drives the development. Could you tell me more about that?

Maronpot: I think that when there's a new tool somebody's got, they typically have some preliminary information that really looks good. I'll use a transgenic example.

Shostak: That would be great.

Maronpot: So, an investigator at the Baylor College of Medicine had a transgenic animal that was supposedly a prostate cancer model that was called the TRAMP model, t-r-a-m-p (Transgenic Adenocarcinoma of the Mouse Prostate). He claimed a certain number of things about this model that he had engineered; that it had prostate lesions like humans and therefore was going to be a useful model. So I obtained a material transfer agreement and got some animals here to use because we just didn't have a very good model for cancer studies regarding prostate. There just weren't any mouse prostate cancer models at the time, and this one seemed reasonable. And so in the meantime, of course, he had a head start on everybody because he developed the animal and he was publishing his results; he was trying to get his grants so he could continue his work. So there's a tendency to promise a little bit more or to be extremely optimistic, and I presume that he was. And so he would describe what he was seeing in these animals in the most favorable terms that would indicate that the animal basically had prostate cancer like humans have. Well, when we finally got the animals here and built up a colony and began to study the TRAMP mouse, we realized a number of things, some of which I knew before and some of which were surprising. One is that the rodent prostate is not at all like the human. The rodent has several lobes of the prostate, and in the human it's all one tissue mass. These different lobes responded in different ways. No matter what you did to these animals, they were going to develop prostate cancer pretty rapidly because the transgene that was used to make these animals was so powerful that it just turned all the cells cancerous. We began to characterize the lesions over time because we now had the animals and we were trying some experiments. We didn't know at first that this was probably not a good model, at least from the viewpoint of NIEHS. You can't test chemical agents in an animal to see if they produce cancer if the mice are going to get cancer rapidly on their own. And so the only logical thing to do at that point was to see if we could do something to prevent it or slow down cancer development.. And that proved extremely difficult, again, because the transgene that was used was so powerful that we could slow the cancer development down a little bit, but we could never really stop it.

We've done a number of publications, some of which we're still doing, characterizing the model. It had never really been terribly well characterized. It was developed by an investigator who is a molecular biologist, and I wouldn't have expected that he would have had expertise to characterize the structure (morphology), and that's what's important in the end. You want to be able to diagnose what you've got. This end up requiring pathology, and he certainly wasn't a pathologist.

So it turns out that it might be a tool to put in your toolbox. I abandoned using it for NIEHS needs and went to something else. It was a big disappointment. But it took three or four years to figure that all out. So, I was disappointed because the hype was there, and the promise and it just wasn't well worked out in advance.

The individual in question has gone on to develop other genetic engineering constructs too. I think he also probably realized also that the original model had its limitations.

Considering the present-day situation. Microarrays, toxicogenomics, proteomics, and related technologies are very appealing because the promise is so great, that more and more people are buying into the technology as well as the associated informatics. Thus, we are being pulled along by the technology - trying to learn it and wrestling with the variables so that we can make them constant. We are not yet at that point. In fact, I don't think we are really yet at the point where we have a clear idea of the question that's being asked and how best to get an answer to the question. Right now we're still wrestling with the technology, which is pulling us along. And that's sort of an evolution, I think, of how most of these new technologies evolve. The seminar I just attended was on proteomics and identifying numerous proteins in the serum, which sounds very appealing. Taking a blood sample, getting the plasma or serum, and looking for biomarkers, is so appealing, but it's extremely complex. At this point in time, no one has answered a relevant question, but the potential for this approach is extremely appealing. We're trying to learn the technology and trying to understand it and see if it'll deliver on the promise.

Shostak: So let me back up a little bit and ask some very preliminary questions about when you came to NIEHS and to what laboratory you came.

Maronpot: I came in 1981. It was called the Pathology Branch at the time, and I came into a new position that was created. It was called the Experimental Pathology Group. What I came here to do was to tease out the underpinnings of some of the responses that were seen - pathology responses such as cancer or toxicity-in the studies that were being done by what was then the newly formed National Toxicology Program. I had laboratory facilities and technicians to chased after the important questions for which the National Toxicology Program wanted answers.

Shostak: And can you map for me the . . .

Maronpot: What happened after that?

Shostak: Yes.

Maronpot: Yeah. It's sort of interesting. There were several reorganizations of the Institute with respect to intramural research divisions, and the National Toxicology Program was always carried along in some way with and affiliated with one or another portion of the intramural research component. Somewhere along the line, two pathology groups were formed. One was called the Pathology Branch, which dealt with supporting the National Toxicology Program, basically make sure the diagnoses are right and that the pathology was well done. And my group was called the Laboratory of Experimental Pathology. It was created as a DIR laboratory, and while we still pursued questions of interest to the National Toxicology Program, it wasn' t limited just to that. There was also a lot of collaborative support pathology-wise for the intramural investigators who were not pathologists but needed pathology support. So there were these two pathology groups.

At the last reorganization that occurred, which was probably five years ago or so, it was decided to put the two pathology groups back together And so, we now have one group, the Laboratory of Experimental Pathology, and it has a dozen pathologists, most of which support the National Toxicology Program. As the newly merged pathology group evolved, it became apparent to me that what the Institute needed was not another laboratory chasing after its own research questions, but, rather, that the Institute needed pathology support in the form of core laboratory support, not just for the National Toxicology Program, but for the entire Institute. And so we created a series of core laboratories and became basically a service organization. Since we're really good at pathology, the advantage to the Institute is that it gets that expertise. The advantage to the pathologists is we get to work with all our really good investigators, usually in a very collaborative fashion. The only thing that was retained from the old days was there one group in the laboratory where there was a PI. The rest of the Laboratory of Experimental Pathology is comprised of staff scientists and technicians. So we're basically a support organization at this point, with the exception of that one lab. It's a small lab. So that's where we are today. We're part of DIR. My immediate supervisor is the director of Environmental Toxicology Division, which basically houses the National Toxicology Program plus some research laboratories.

Shostak: Right. So is that Chris Portier?

Maronpot: Yeah, it's Chris. But then, for purposes of space and personnel, I report to Dr. Birnbaumer. Chris does my performance review. If I want anything like budget or space or people, I deal with Dr. Birnbaumer, typically with support from Chris.

Shostak: How did transgenic mice come into your overall research program?

Maronpot: The concept of using them originated within the Institute. It originated down in Ray Tennant's group, perhaps mostly with the Tg.AC mice that he had. I think that it was based on a collaborative effort he had with the investigators at Harvard, where they discovered quite by accident that these animals developed skin cancers pretty easily just by wounding them. Ray has been a very visionary sort of individual, and so he could see the potential that this model and models like it would be potentially useful as a quick way to screen environmental agents to see if they had carcinogenic potential. About the same time, but elsewhere, the p53 knockout was being developed. So there was these two genetically engineered mice, at least initially, that were about equivalent in terms of their evolution or what we understood about them. The appeal of the p53 knockout was that it made sense relative to human cancers, which usually have alterations in the p53 gene. The Tg.AC, although developed for an entirely different purpose, fortuitously seemed to show reasonably rapid response, and Ray was finding out that if you painted things on the skin, you would get a pretty rapid response. So he proposed that they might be considered it as a short-term bioassay.

At first, the talk was to replace the conventional two-year rodent cancer bioassay, but I think as time went on, it was realized that we couldn't really dislodge that paradigm very easily. So it was then proposed as an adjunct or possibly an early screen.

Ray was doing in-house experiments at the same time that he was proposing that the National Toxicology Program adopt both p53 knockout and the Tg. AC mice. And because he was doing in-house studies, at some point in time it became apparent that he needed some pathologic diagnoses, and that's where the pathology group entered the scene. We began to realize a variety of things. First of all, nobody had any idea what the background pathology was on the FVB mouse, and that's what the Tg.AC was built on. And so we realized that we needed to work that out. Transgenics were the little darling of those times, and some of the in-house investigators were creating their own for their research purposes, and they also didn't know what the background incidence of lesions was in the strains they were using. So we decided that we should establish lesion incidence in background strain animals that we would let age out normally just to see what they got spontaneously. Sometimes if you manipulate a gene, all you're going to do is cause what they're going to get normally to occur earlier, and that's okay if you understand that. Or, alternatively, maybe these mice are going to develop a novel response, and that would be very indicative of whatever the question was you were asking. Knowing the background lesion incidence would allow us to identify lesions as novel or just background. So pathology entered of the scene simultaneously to support in-house investigators and to help Ray Tennant get some pathology done on those animals. Ultimately, as the National Toxicology Program began to use these animals in the hopes that they would be a quick bioassay cancer screen, all the NTP pathologists needed to get involved to standardize the pathology and make sure it was done right.

In parallel, the Japanese were developing a transgenic animal that they specifically believed would be a relevant six-month screening bioassay, because they put a human *ras* gene into their mice (*ras*H2). It was actually a normal human *ras* gene. And that had more logical appeal than anything else because it would have a human gene that could be altered by treatment. It would have the mouse gene, also, as that didn't go away. The preliminary findings were that the *ras*H2 animals responded very rapidly, and you didn't need very many animals.

So I engaged in a collaborative effort early on to do an interlaboratory variability study on these animals. There was always the question that if we got the animal and did the same kind of study as the Japanese scientists were doing, would we get the same answer. And so we picked some human and mammal carcinogens, about a half a dozen, and they shipped me some animals to test. We did the study here and simultaneously they were doing the same thing in Japan, so that we could look at interlaboratory concordance and variability. And that's when I really got into the transgenics personally. No one here had that particular transgenic animal, and it had a lot of appeal from the scientific point of view. This model was appealing because the Japanese created it for the purpose of doing cancer bioassays whereas the others transgenics under cnsideration were fortuitous, having been initially established for other purposes.

The interlaboratory variability was reasonable. We had our usual problems with using new kinds of animals in new ways. This was done in a contract laboratory locally. And all sorts of things we had never considered using some of the known human carcinogens occurred, like issues of solubility and stability that we really hadn't thought much about since we were so keen on testing in these unique animals. A couple of errors were made in the contract laboratory, some experiments needed to be repeated, but the study was eventually completed and reported in the literature.

Thus, there were three or four, if you consider the model proposed by the Dutch scientists that were the focus of attention as potential cancer bioassay models.

Shostak: What's the one engineered by the Dutch?

Maronpot: It's called XPA, and it's a knockout. They've actually crossed it also with the p53 knockout recently. It hasn't been tested in as many chemicals. It's sensitive to ultraviolet light.

Shostak: The XPA model?

Maronpot: Yes, because it can't repair the DNA lesions that are produced when exposed to ultraviolet light. The Dutch are proposing it as a short-term mode. They just haven't done as many chemicals.

Shostak: So the models that you've worked with are Tg.AC, p53, and *ras* H2, and TRAMP.

Maronpot: Yes. And, locally at NIEHS, other research models such as the ERKO and BERKO, alpha and beta estrogen receptor knockouts and COX knockouts, as examples. These days, as a collective group of pathologists, we are helping to phenotype some of these genetically engineered animals for in-house investigators. We have about five phenotyping efforts ongoing. I just finished one last night. I didn't find anything wrong with it, which is important.

If you think you've got a mouse that's going to be useful for some purpose but you don't want it to have unintended background changes, then you need to get all the tissues looked at to make sure that everything is normal. The one I just finished was a p450, or a 2C29, that the investigator asked me to look at; I looked at all the major tissues, and they're all basically normal. So I think they're going to be happy about that. I don't think they wanted something else to be going on in these mice. There's a DNA repair-deficient knockout that Dr. Sam Wilson and Rob Sobol are working on. We just sent a prosector up to Detroit to, as a collaborative project, to necropsy and collect the tissues from some of Dr. Wilson's genetically engineered mice. I think the tissues are probably due to arrive today, and then they need to be processed and turned into slides. And one of my pathologists is going to do the morphological phenotyping on those mice.

Dr. Wilson's postdoc, Rob Sobol, who had beta-pol overexpressing mice, and we are phenotyping some of those animals. These were animals that were allowed to age out. Basically all they have of note is cataracts. But that's important if it's a good model for cataracts

It's sort of a pathology support, but it goes beyond just diagnosing each of the slides, because we end up helping them do laser-capture microdissection and immunohistochemistry and, in the near future, *in situ* hybridization.

Perry Blackshear has got some knockouts he calls B11 that get brain defects, so we've been trying to understand why they're dying *in utero* by looking at 11-day embryos. Actually, the naming of some genetically engineered mice isn't always intuitively obvious, although ERKO (estrogen receptor knock out) and BERKO (beta estrogen receptor knock out) represent examples of where the naming has some intuitive appeal.

Shostak: You've mentioned a couple of times having to get the animal sent to you from Japan, or Ray Tennant's lab is working with the folks up at Harvard. What is the process of kind of getting the animals in-house, and has that been challenging in any way?

Maronpot: It's challenging, but not overly so. You've got to have material-transfer agreements. Actually, first of all, you've got to talk to the investigator, and you obviously can't have a commercial notion in mind, but being in the government, that doesn't seem to be a problem. Because we are a government facility, it's pretty easy to get an investigator to agree to share his mice, particularly because the implication is there that it would be a collaborative endeavor and most investigators want to collaborate with governmental laboratories. And if they really think their model is useful, they like to see people use it. So you get a material-transfer agreement, which doesn't take too long for us. And then you wait and wait and wait to get the actual founder animals in. So the TRAMP is a case in point. I got the paperwork done pretty quickly. It took two years before they sent me two animals. In the case of the TRAMP mice, many scientists were asking for animals. While it is relatively easy for the investigator to agree to provide animals, the delay seems to relate to the technicians actually breeding the mice. Either the word doesn't get to them to readily and/or they are busy generating mice for their own purposes. . Ultimately, they sent me two animals, two females, and so I had to get them bred and get offspring and genotype them, followed by more breeding to build up a colony. It was at least two years after the initial discussion with the investigator and his agreement to actually have enough animals to even begin to use them. That's a difficulty, but it's not insurmountable. Fortunately we had access to contractors to actually do the breeding. We couldn't produce enough mice ourselves once we got the initial two females. And getting them on the right background sometimes is another case in point.

There's an interesting knockout animal called the CAR-KO. CAR stands for constitutive active receptor. It's a nuclear receptor mouse that Dr. Negishi wanted to use. I've been working with him now for a good year, if not longer. He came to see me because he wanted to see if this animal model would be useful to answer his scientific questions, and these involved generation of liver tumors. He indicated he could get the animals from another investigator. He had to get a material-transfer agreement, and, fortunately, this time we didn't have to wait as long. I recall that in six or eight months, maybe 10 months, we got about 6 mice. And so I put them in the contract lab that I'm the project officer for this individual, and the idea was to breed them to have enough for studies. They came on a C57 black background, and he wanted to have a model where we could generate liver tumors because the idea was that these animals wouldn't get the liver tumors because of the gene that was knocked out. Since they were on a C57 black background, and C57 mice aren't susceptible for liver tumors development, we needed to put them on a susceptible background for liver tumor development. We also didn't know anything about this knockout in terms of its tumor susceptibility. It really hadn't been characterized, and still hasn't been characterized. We put it on a C3H background and we're going ahead doing the experiments, even though we really don't know the natural history of disease in this particular knockout on a C3H background. Getting them on the C3H background took a year and then we did the study. The results are fantastic. In the process, we will also generate data on the natural history of disease in the CAR-KO on the C3H background. The investigator is delighted. The experiment has been underway for 6 months with a few more months to go. This has been a short enough duration for a study to satisfy the investigator.

This CAR-KO experiment has been exciting. It's exciting because it worked really well, but also because of the importance of this particular gene that has been knocked out. I'm looking forward to seeing the diagnosis and seeing them get their paper out, even though there will be a large number of slides and Dr. Negishi will want the results promptly. So, I'll probably have to spend the weekend evaluating the slides.

Shostak: That's very kind of you.

Maronpot: Well . . .

Shostak: Weekends, of course, being weekends.

Maronpot: Weekends are weekends. Right, yes. Saturdays and Sundays, and fortunately the kids are all grown and out of the house, and I'll manage.

Shostak: I would like to take a break for one second, if I may, and then come back and ask you more questions.

Maronpot: Sure.

[Tape recorder turned off.]

Shostak: Okay, now it's on.

Maronpot: Okay. So, there's two aspects to the use of transgenics in which I've been involved, along with the other pathologists here at NIEHS. The first is are these useful models for identifying carcinogenic potential as a screening bioassay? For this purpose, they haven't turned out to be as good as many had hoped. The second aspect is their use as a research tool. As research tools, when you're talking about the BERKOs or the CAR mice, these are extremely powerful models, extremely powerful tools, and we have a really good situation when we can use the right tool for the right question. For example, you wouldn't use a BERKO for looking at p450 enzyme metabolism; you'd use the CAR. And you wouldn't use the CAR for some of the things that Ken Korach does with the BERKO and estrogen receptor. So I think as a research tool, these are phenomenal animals and will always be, and scientists will keep developing new ones. So I just wanted to make sure that was pretty clear. I'm a little bit under whelmed by the response that I've seen when we've considered these animals as bioassay animals in identifying carcinogenic potential. But as research tools, you can probe very specific questions, so they have great added value to biomedical research. And as pathologists, we like looking at them all anyway, so . . .

Shostak: Why is that?

Maronpot: Because they have unique lesions and multiple lesions, and so there's nothing more boring for a pathologist than looking at a bunch of normals. These genetically engineered mice often have unique tissue changes and are thus more interesting to a pathologist.

Shostak: When I started this project, I had a meeting with Ray [Tennant] and with John Bucher and with Mary Wolfe, and one of the things that they stressed that I ask you about specifically was the collaboration with the researchers in Japan, because I understand that that was kind of a unique situation.

Maronpot: Yes. It was unique because there were some logistical considerations to get the animals here because they wouldn't allow us to breed them. They produced them all. And shipping from Japan is a non-trivial exercise, particularly in certain times of the year. The first stop from Japan for the mice is Seattle. It can't be too cold there or the animals can't be shipped. If they next get transshipped to Texas on the way here, it could be too hot for shipment. So, they would produce the mice and hope that when it was time for shipment, weather conditions along the way would be suitable. It would not be appropriate for the animals to sit at a hot airport while the paperwork gets cleared through customs. Since the shipment time was long, they used potatoes, cut potatoes in the transfer cages, as a source of fluid and food. And they all survived; we never lost any. We had some cancelled flights because the weather was bad, after they had prepared a shipment of animals for us. It was all highly orchestrated because the mice needed to arrive here at a certain age so that we could start the experiments at an appropriate age following the acclimatization period. That means they had to breed them and to have enough ready for us - males and females. And then if the flight got cancelled, the mice would be too old to ship later and they'd have to start all over. So we had a lot of back-and-forth logistics going on to make it all work. And that was unique. What was also unique is that they came over and approached me to see if I would be interested in this collaborative effort.

Shostak: I was just about to ask that.

Maronpot: I have a feeling it's because of the mouse pathology part. We're really good at rodent pathology, and that was known even before we published our mouse pathology book.

Shostak: Okay.

Maronpot: Although this [the book] wasn't out at the time, our reputation was out there. Also we had done a rat pathology book a long time ago.

Shostak: I met Gary Boorman the last time I was here. He's lovely.

Maronpot: Yes. He did the rat book - he and some others in the early 90's.

So I think that's why the Japanese approached us, because we have a good reputation as a collective group of pathologists - more knowledgeable of rodent pathology in the aggregate than probably anywhere else in the world. I just don't think there's such an aggregate of people. So I suspect that's largely why they came over. Since I had been to Japan a number of times and given talks, I think my name was known, so they contacted me and came over. And the guy that heads up the laboratory that produced these animals and generated them was in his mid-seventies when he came over, and you wouldn't know it, very energetic. And we just had phenomenal rapport, so that helped, and he was the kind of person that, when something was decided, it was to get done, and he's very efficient. That helped make it work, and that's sort of is in sync with what I like to do. Let's get it done, not just talk about it. And so that worked for both of us because our personalities were compatible. It turned out to be a reasonably useful animal, and now is available in the States, so you don't have to get it from Japan anymore. You can buy it commercially in the States.

Shostak: Is it available from Taconic?

Maronpot: Yes. They entered into an agreement with the Japanese to get it over here. So it's probably the best of the three favored genetically engineered mice proposed for cancer bioassay screening, but not perfect for bioassay screening. And so the collaboration was really good, and we wrote a paper. I didn't need to do anything more, although I offered to do a few more things for them. I think they weren't as keen on doing more because they already achieved the payoff of having an interlaboratory comparison. What I offered was to characterize this animal completely - to do the hematology and the clinical chemistry, to let them age out so we could define their spontaneous disease patterns.

Shostak: Can you tell me more about the *ras* H2 model? You said it was being evaluated as a short-term bioassay -- is it also a model for a specific disease?

Maronpot: No, I don't think it was designed as a specific disease model. I think it was designed for short term cancer bioassays only for that purpose. Initially they tried different chemicals that would be expected to produce certain target-organ cancers, so the model had promise. Like other genetically engineered models that have been proposed for bioassays, they have their own intrinsic pathologies, tumor pathologies. So in the case of the *ras* H2, because of the background strain, they get some lung tumors and they get hemangiosarcomas of the spleen. Splenic hemangiosarcomas are not a usual bioassay response. We generally expect liver tumors or some other epithelial cancers, aside from the lung tumors, although they get occasionally develop skin tumors. When treating these mice with either a known or unknown carcinogen, what you get is more of what they spontaneously develop more lung tumors or more hemangiosarcomas of the spleen. If you treat them with a liver carcinogen, you would expect liver cancer based on what's been determined in other animals; however, using this and similar transgenic models, you often don't get what you were expecting. However, if you give them a brain carcinogen and all you get is hemangiosarcomas in the spleen, that doesn't mean it's not a good model; it's just not a good model for brain tumors (or liver tumors).

Shostak: Right. One of the other things that was suggested to me that I ask you about was the prostate cancer models. I assume that was the TRAMP model primarily.

Maronpot: Yes.

Shostak: But could you talk to me a little bit about the process of developing models for specific disease, other target organs or outcomes?

Maronpot: I don't have hands-on experience in generating these models, but just by working with all these various genetically engineered models, I have a notion of the process. The investigator first inserts something or remove something from the genome that makes biological sense. So in the case of the TRAMP model, they decided that they would use a very strong transgene, the SV40 transgene, which, if it could be directed to tissue X, you will get cancer in tissue X. That particular transgene has been used a lot. It's extremely powerful. And that was my concern about the model. You couldn't slow down the process because the transgene was so dominant in its effect. In the case of the TRAMP, they needed to find a way to direct it to the prostate. They already decided they were going to use the SV40 transgene. And they decided they would use what is called a promoter, in this case the rat probasin promoter. At the time, they knew that probasin was produced in the dorsal prostate of the rat. It was a protein, and its function is not clearly known even today. So they inserted the rat probasin promoter basically in front of the transgene, and inserted it into the oocyte pronucleus of the animal. That's kind of really oversimplified. During growth of the embryo, it went throughout the body, but it only worked in the prostate because that's the only place that the probasin promoter was active and made any sense biologically, I guess, to the animal. And so, lo and behold, they found they got prostate lesions, plus some other lesions which weren't prostate, as we found out later. However, all the lesions were in the accessory sex glands of the male. Since lesions were not seen elsewhere in the body, I think that sort of encouraged the development of this model.

So, for something like the TRAMP model, someone came up with something that made biological sense, and that's a good example. The Tg.AC was entirely fortuitous. It was developed for a different purpose involving the fetal globin gene, and in using the animals, it was discovered by accident that, when some animals injured themselves in the cage, they developed skin tumors.

The p53 knockout was designed because p53 is a common, probably *the* most common tumor-suppressor gene alteration in human cancers, and so that made sense. Investigators wanted to see what was going to happen if this gene was silenced in mice. I think the question was a basic science question, rather than an initial interest in developing a bioassay model. The human cancers where p53 seems to play a role tend to be epithelial cancers for the most part. The p53 knockout, possibly because of the C57 background, tended to die with lymphomas, which are not epithelial. So this mode did not spontaneously gett epithelial cancers. Nevertheless, it made some sense that maybe we could identify potential human carcinogens by using this model. That's why they tried it as a bioassay model. So there's a little bit of biology behind that.

There was a little bit more biology behind the development of the *ras*H2 model. They knew that the *ras* oncogene was activated in a decent number of human cancers, not all, not as much as p53 played a role. They also knew that the *ras*H2 oncogene was -- or proto oncogene, really -- was important in rodent tumors. So they thought, why don't we just put the human *ras* gene in the mouse, attempt to humanize the mouse to that degree, with its own endogenous human promoter. They were basically trying to humanize some little segment of its genome. And the idea then would be, if we can produce tumors in this animal by any fashion by giving them unknowns, we can look for the point mutation profile in the *ras* oncogenes, not only in the mouse ones but in the human one as well. So they had in mind to do a bioassay and to sort of humanize the mouse. *Ras* was easier to do. P53 is a knockout; with *ras* you're adding something to the genome.

Shostak: Knocking in?

Maronpot: Not a knock-in. It's a transgenic.

Shostak: Okay.

Maronpot: It's an insertion. So it was easier to do technologically. They produced it for bioassay purposes, and then when they tested it with really strong, known carcinogens, it really worked nicely. So they were propelled to move forward and espouse this new model. And shortly after that, I think, they contacted me. Partly also, as I think back on it, it isn't just because we know mouse pathology. I think it's also because it's the National Toxicology Program, and it's got a pretty good reputation. So if you can collaborate with the NTP and you're trying to develop a mouse in Japan that you think ought to be useful and have visions of seeing a globally important, why not? We're impartial and it's not going to hurt you the least bit.

Shostak: Right. As you said a moment ago, why not aim high?

Maronpot: Yes, right, exactly.

Shostak: Did you participate in the ILSI Alternatives to Carcinogenicity Testing Committee?

Maronpot: No, I didn't. We had enough people from here participating at the time. Before they actually formalized what they were going to do, I was at a couple of meetings. I was a little bit disenchanted with some of the participants, whose interests I thought were self-serving, and I just didn't -- I had plenty of things on my plate and just didn't really want to participate in it, also realizing it would require lots of meetings. I have a difficult time in some meetings when there is a lot of posturing and a lot of compromise. When you get together and you've got to deal with people that are doing all this posturing and so on. And I just didn't feel I needed that. At that stage, I had plenty of really interesting things to do, and there were plenty of people that were participating not only from here, but elsewhere, and people with good reputations. So I decided not to do it, but I certainly followed it very closely. And I edit the journal *Toxicologic Pathology* and we published the outcome as a special issue of that journal.

Shostak: I have your intro to that issue.

Maronpot: So we, I mean, I've certainly been very interested in it.

Shostak: Can I ask you then, just kind of generally, what your perspective is on what the committee actually did or accomplished?

Maronpot: Well, I think they did what was necessary. They attempted to, in a global way, which is important in terms of international harmonization because we're in a global situation here - they attempted to validate these models to some degree, within the constraints of the resources that were available. There were considerable resources, collectively, that were put into this.

I think what they might not have realized at the time was that to validate a cancer bioassay model involved validating a model of a very complex disease. We knew from previous experiments with various models that they typically do not predict well, but the potential offered by these genetically modified was hard to resist. In a sense, the technology was pulling us in a specific direction – the promise was too great to pass up. And, if it had worked, that would have been super. But it didn't or at least, not according the expectations. The approach has evolved a lot since then, and I don't think anybody believes a six-month study with a limited number of animals, even though they're transgenics, is the right way to go. Exactly what is needed, say more animals and a 9-month duration, would require its own validation, although we could probably get by a little less vigorously on a second time around. I doubt there will be another orchestrated international validation attempt with genetically modified mice in the near future. At the end of the day, the problem with the overall effort was that they couldn't get enough chemicals to paint a broad enough stroke across the bioassay template to understand if the models were really good. They didn't know if they needed to use one model or two, and, if two. So, in the end, there wasn't a clear picture. If it had been more clear, I believe you would be seeing additional ongoing studies. In the meantime, I believe the NTP has decided to put transgenics on the back shelf in terms of cancer bioassays, or, as I like to put it, in the toolbox, to be removed and used when needed to answer a specific question. And I believe, in large part, the ILSI folks have done the same thing, with reluctance. I proposed a new model at about the time when this was going on. It was the p27 knockout. This mouse develops numerous epithelial tumors that are like human tumors, including prostate tumors. p27, like p53, is important in human cancer development. Because the investigator who was promoting this mouse as a potential bioassay model had convinced me that it had advantages over those previously used in the ILSI study, I suggested he write a prospective piece for the journal *Toxicologic Pathology* in which he could highlight the virtues of the p27 model. And he agreed. I sent it to 8 to 10 reviewers because I felt it would be controversial in light of the ILSI study outcome. Included were 5 or 6 internal NIEHS people, none of whom responded. I think that the appeal of using transgenics had worn thin and that the timing was just wrong to consider another foray into that arena.

Shostak: That surprises me.

Maronpot: I did get answers from six outside reviewers, since this was a journal article going out for peer review. The response was 50-50. Some people loved it and some people hated it. We published it anyway. That's why it ended up being a perspective since it was the authors' opinion.

I still think it has potential as a model based on the biology, and, if the NTP decides to revisit this arena at a later time, I will recommend that the article be read for consideration of the proposed p27 model. The p27 model can be put on a B6C3F1 background, i.e., the mouse background used by the NTP and for which we have good background data.

Shostak: I have just a couple more questions for you. I read an article that you wrote with Gary Boorman -- it was published in '96 -- about the importance of the mouse in evaluation. One point that you make in that article that was really interesting to me is that one needs to distinguish between the kind of information that goes into the review of pharmaceuticals and the amount and the kind of information available to evaluate environmental chemicals. When I read that, the first thing I thought of was the International Conference on Harmonization statements, which were very much oriented towards the evaluation of pharmaceuticals.

Maronpot: That's right.

Shostak: And it seemed to me like you were saying, "Okay, yes, but environmental chemicals are different."

Maronpot: Are different, right.

Shostak: Did I read that correctly?

Maronpot: Yes, you did read that correctly. And there might not have been enough information in there to understand why we made that statement. In the development of pharmaceuticals, there's a lot known about the compounds. There's a lot known because it's possibly been created based on similar but known compounds. Drugs are tested rather extensively in rats, and a lot is learned about their pharmacokinetics, tissue levels, and metabolism. A lot of data is generated before the pharmaceutical company will take the step of doing a cancer bioassay. So, knowing that much information in advance, one can make an intelligent decision about how important it is to test in two species. FDA has been receptive to considering cancer studies in rats and in a transgenic mouse as the second species and this is in agreement with one of the recommendations in the ICH document for pharmaceuticals.

Pharmaceutical companies have found this useful because they didn't have to deal with the two-year mouse bioassay and the costs and they can get a quicker answer. They could actually do the mouse transgenic study first if they really wanted to, and, in parallel, start the rat study. I didn't have any problem with this ICH approach, because I would assume that pharmaceutical toxicologists are using all their knowledge to make a judgment about how to do cancer testing.

On the other hand, we know very little or nothing about the toxicity/carcinogenicity of new pesticides and environmental agents to which we are exposed. Typically, they're unknowns; that's why we're testing them. And even with some toxicokinetics that we might do, we are still testing these compounds as unknowns. Being conservative, we tend to default to what we have been doing on so many past bioassays.

Shostak: That's helpful. Thank you. Just one more question, and you've already alluded to this, could I ask you to speculate a bit on the future of transgenic models and environmental health research.

Maronpot: I think they'll be used for basic science type questions, basic biology, be it cancer or maybe something else other than cancer, like degenerative diseases for example. And I think they'll have a very useful role in that regard, in teasing out the underpinnings of whatever the disease process is the investigator is going after. I don't see them reemerging on the cancer bioassay short-term model stage again, although it might come around in specific cases that would make sense. I believe that transgenics will be basic science research tool.

Shostak: Do you think -- and I realize that this is somewhat speculative as well, but, and you're describing a kind of science moving forward in part, be it by this process of being technology driven. Do you think there are any detriments to having science develop through that process?

Maronpot: No. There's not necessarily a detriment. But there's a concern that the descriptive empirical morphologic evaluation of the tissues is not considered terribly important. But it is actually vitally important, so that I see the need to constantly remind folks of the importance of getting a phenotypic anchor that is tissue specific for each significant molecular change they find. You need to know what it is that you're analyzing with these new and extremely powerful technologies.

Generally morphology is not highly regarded because it's subjective; it doesn't have a number associated with it. Interestingly, when it's time to publish a paper, these same folks want a photomicrograph to show the lesion, and they come to my door. What I'd like to convince them to do is, in the beginning, to be careful and certain about their sample selection and know what it is that they're getting.

Shostak: Let me ask you one more very general question, which is just, is there any aspect of this story of transgenics at NIEHS, at NTP, that we haven't touched on that you would call my attention to?

Maronpot: No, not really, other than, not surprisingly, as you would probably imagine, it's very expensive, but so are the newer technologies, even more so. No. I think nothing I'm aware of right offhand.

Shostak: Okay. I will turn this off.